



Protocols for Decontaminating Biomonitoring Sampling Equipment



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**Bureau of Land and Water Quality
Division of Environmental Assessment**

**Standard Operating Procedure
Protocols for Decontaminating Biomonitoring Sampling Equipment**

- 1. Applicability.** This standard operating procedure (SOP) applies to all equipment (sampling devices, nets, boats and trailers, etc.) used in the collection of biological samples from waters of the state of Maine that come in direct contact with the waterbody. This SOP applies to biomonitoring activities conducted by the Division of Environmental Assessment (DEA) in all freshwaters of the State of Maine, unless more stringent program or project decontamination protocols already exist. This SOP also applies to all non-DEA entities that conduct biomonitoring activities within the State in cooperation with DEA staff.
- 2. Purpose.** The purpose of this SOP is to provide standardized methods for cleaning and disinfecting all equipment used to collect samples from waters of the state of Maine in order to prevent the spread and/or introduction of disease pathogens and invasive algae, plant and animal species. This SOP is designed to be consistent with the decontamination procedures of other entities conducting sampling in the State, such as the Department of Marine Resources, Inland Fisheries and Wildlife and Acadia National Park, as well as the procedures of the Vermont Agency of Natural Resources.

Examples of confirmed and potential disease pathogens of concern in Maine include, but are not limited to: Chytrid fungi, *Ichthyophonus*, Ranavirus, *Ribeiroia*, *Saprolegnia*, *Proteocephalus ambloplitis* and other fish pathogens. Examples of invasive aquatic plant and alga species of concern in Maine include, but are not limited to: Variable water-milfoil (*Myriophyllum heterophyllum*), Eurasian water milfoil (*Myriophyllum spicatum*), Hydrilla (*Hydrilla verticillata*), Curly-leaved Pondweed (*Potamogeton crispus*), and Didymo (*Didymosphenia geminata*).

3. Definitions.

- A. Chytrid fungi. Ubiquitous fungi that inhabit aquatic habitats and wet soils, infecting plants, algae, protists and invertebrates. *Batrachochytrium dendrobatidis* is a Chytrid fungus known to infect vertebrates, including amphibians.
- B. *Ichthyophonus*. A protozoan-like organism which affects marine and freshwater organisms. *Ichthyophonus* infects the skeletal muscle of amphibians, and has also been identified as the cause of herring die-offs.



- C. *Ranavirus*. A common virus in New England that primarily affects wood frogs and spotted salamanders. This pathogen was associated with a large die-off of spring peepers in Acadia National Park.
- D. *Ribeiroia*. A common pathogen which causes malformations and deformities in amphibians belonging to the genus *Rana*.
- E. *Saprolegnia*. A pathogen which affects the eggs of many amphibians.
- F. *Proteocephalus ambloplitis*. Fish pathogen, commonly known as bass tapeworm.
- G. Variable water-milfoil (*Myriophyllum heterophyllum*). Invasive submerged aquatic plant, non-native to the Northeast U.S., that out competes native species and clogs shallow areas of lakes, making swimming and boating difficult.
- H. Eurasian water milfoil (*Myriophyllum spicatum*). An invasive submerged plant with feather-like whorled leaves. It grows in extremely large dense mats in depths up to 15 feet. This plant produces emergent flower spike important for distinguishing Eurasian from Variable-leaf and other milfoils.
- I. Hydrilla (*Hydrilla verticillata*). A prolific invasive weed that forms stems reaching up to 30 feet in length, dominating fresh water ecosystems quickly by way of winter buds, underground tubers, and surface runners. It is tolerant of low light levels, high or low nutrient levels, and freezing temperatures, posing fouling problems for swimmers and boaters.
- J. Curly-leaved Pondweed (*Potamogeton crispus*). An invasive submerged aquatic plant from Eurasia. It is highly competitive plant, spreading and growing rapidly. This plant grows in cool waters and spreads by rhizomes, turions (vegetative buds) and fragmentation. Of particular concern are the turions, which are released from the plant in late spring and float until they sink to the bottom where they lie dormant until water temperatures cool enough for sprouting in the fall.
- K. Didymo (*Didymosphenia geminata*). An invasive freshwater diatom species, also known as Rock Snot.

4. Responsibilities.

- A. Training. It is the responsibility of the Project Manager to ensure that all individuals collecting samples have received training in and follow these decontamination procedures.
- B. Determination of threat. It is the responsibility of the Project Manager to determine if a significant threat of contamination exists, which level of decontamination is necessary, at what point in the sampling process the decontamination should take place (before sampling, after sampling, or before and after sampling), and what disinfectant should be used.



- C. Out of State Equipment. It is the responsibility of the Project Manager to ensure that all cooperating entities follow the Level 3 Cleaning and Disinfection procedures, see 5 C (3), below.

5. Guidelines and Procedures

A. Materials

- (1) Disinfectant. **For non-absorbent materials** (boats, rubber waders and other “hard-sided” objects), use either a 2% household bleach solution (3 oz bleach per gallon of water) or a 2.5% Quaternary ammonia (Sani-Care Quat-128, etc.) solution (3.5 oz quaternary ammonia per gallon of water). **For absorbent materials** (nets, felt-soled waders, life jackets, sandals with fabric straps and other “soft-sides” objects), use a 2.5% Quaternary ammonia (Sani-Care Quat-128, etc.) solution (3.5 oz quaternary ammonia per gallon of water). Note: an alternative disinfectant can be used, if approved by the Project Manager.
- (2) backpack sprayer, garden hose or other suitable applicator
- (3) scrub brush
- (4) liquid dish or hand soap (phosphate-free and biodegradable)
- (5) measuring cup (with cup and ounce increments marked)
- (6) plastic container(s) large enough to hold items that need to be soaked
- (7) plastic bucket (to rinse small items)
- (8) 5 gallon plastic container of tap water
- (9) rubber gloves (including an extra pair)
- (10) goggles
- (11) plastic apron (optional)

B. Precautions and limitations

- (1) Always wear gloves and safety goggles when using disinfectant, and avoid contact with exposed skin, clothing, vehicle upholstery and/or other fabric.
- (2) When using a backpack sprayer, keep it upright at all times to avoid spillage.
- (3) New bleach solution must be made up daily. New quaternary ammonia solution should be made up every 2-3 days, or as needed. Old solutions must be disposed of down a drain connected to a wastewater treatment system, accompanied by a large amount of water.
- (4) For safety and logistical reasons, only take one type of disinfectant into the field. It is up to the Project Manager to decide which one will be needed based on the types of equipment to be used in the field (absorbent vs. non-absorbent).

- C. Procedures for inspecting, cleaning and disinfecting equipment. Level 1 decontamination should always be done. The necessity of decontamination beyond Level 1 is to be determined by the Project Manager.

- (1) Level 1 – Visual inspection
 - (a) Applicable to all waters.



- (b) Visually inspect all equipment having contact with the water (waders, nets, sieve buckets, canoe, boat trailers, etc.) for any plant fragments or other debris. If any plant material or associated mud is found, remove it and either throw it in a trashcan or dispose of it on high, dry ground. Do not put it back into the waterbody or along the shore. All plant fragments must be removed before equipment is transported to another waterbody.
- (c) Allow all equipment to air dry and visually inspect again, repeating procedures if necessary.
- (2) Level 2 (done in addition to Level 1) - Cleaning
 - (a) Applicable to: waters used for aquaculture activities, waters within a State owned Ecological Reserve, waters designated Statutory Class A or B, or as deemed necessary by the Project Manager.
 - (b) Visually inspect all equipment having contact with the water for any plant fragments or other debris, as outlined in C (1) (b), above.
 - (c) Designate a grassy area or other upland vegetated area, at least 100 feet from open water and remove mud and other debris, by washing with soap and water. Rinse with clean water; either tap water or de-ionized water, as determined by the Project Manager.
 - (d) Allow all equipment to air dry and visually inspect again, repeating procedures if necessary.
- (3) Level 3 (done in addition to Levels 1 and 2) – Cleaning and Disinfection
 - (a) Applicable to: critical habitats (vernal pools, designated salmon rivers, waters designated Statutory Class AA), areas with a known infestation of Infectious Salmon Anemia virus (ISAV), areas with a known infestation of an invasive aquatic plant, areas susceptible to Didymo infestation, or as deemed necessary by the Project Manager.
 - (b) Visually inspect all equipment having contact with the water for any plant fragments or other debris, as outlined in C (1) (b), above.
 - (c) Designate a grassy area or other upland vegetated area, at least 100 feet from open water and remove mud and other debris, by washing with soap and water. Rinse with clean water; either tap water or de-ionized water, as determined by the Project Manager.
 - (d) Disinfect by thoroughly spraying all equipment with appropriate disinfectant. Bleach solutions are not recommended for absorbent materials due to ineffective penetration compared to Quaternary ammonia solutions.
 - (e) Allow all equipment to air dry and visually inspect again, repeating procedures if necessary.
 - (f) All equipment used to collect water samples (dipper, mixing jugs) must be rinsed 3 times prior to reuse. Rinse with clean water; either tap water or de-ionized water, as determined by the Project Manager.
 - (g) Sampling devices that are placed into a waterbody for an extended length of time (e.g. rock bags, periphytometers) will be decontaminated using one of the following methods, as determined by the Project Manager.



- i. Air dried for several months, in direct sunlight for part of the time, if possible.
 - ii. Cleaned with hot soapy water, rinsed with hot tap water and air dried for several months in direct sunlight for part of the time, if possible.
 - iii. Immersed in a bleach solution bath for 30 minutes, rinsed with hot water and air dried for several months, in direct sunlight for part of the time, if possible.
- (h) This disinfection procedure does not apply to electronic equipment such as dissolved oxygen and pH/conductivity meters. Rinse meter probes with clean water between sites, remove all plant fragments and other debris from meters and cables by hand (clean paper towels may be used if needed), and allow equipment to air dry. Meters and data sondes may be disinfected with an alcohol rinse, if deemed necessary by the Project Manager.

- D. Whenever possible, use non-absorbent, “hard-sided” sampling equipment. The use of rubber-soled waders is preferred to using felt-soled waders.
- E. Whenever possible, dedicate equipment to a single site for repeat sampling. Such equipment must be clearly labeled and kept clean, but does not require disinfection between uses.
- F. Whenever possible, sample upstream sites first and work downstream to avoid potential introduction of pathogens or invasive species further up into the watershed. Uncontaminated sites must be sampled before sites having known disease or invasive species problems.
- G. All equipment used in sites having known disease or invasive species problems must be decontaminated prior to reuse.
- H. All used sampling equipment brought in from outside the state of Maine must be cleaned and disinfected (see C (3) above) before and after its use in Maine waters.

6. References.

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Maine Department of Marine Resources. 2005. *Decontamination Procedures*. Personal communication from Jon Lewis, DMR Aquaculture Environmental Coordinator & Dive Safety Officer.

Vermont Agency of Natural Resources. 2008. *Equipment Disinfection Procedures Required for Aquatic Species and Pathogens Spread Prevention in Rivers and Stream*.



Addendum - List of edits to existing SOP

SOP section	Old text	New text
<i>Edited in April 2008</i>		
1. Applicability	N/A	This SOP also applies to all non-DEA entities that conduct biomonitoring activities within the State in cooperation with DEA staff.
4. Responsibilities	N/A	C. Out of State Equipment. It is the responsibility of the Project Manager to ensure that all cooperating entities follow the Level 3 Cleaning and Disinfection procedures, see 5 C (3), below.
5. Guidelines and Procedures, A. Materials	(1) bleach solution (1/2 cup bleach per gallon tap water) Note: an alternate disinfectant can be used, if approved by the project manager.	(1) Disinfectant. For non-absorbent materials , use either a 2% household bleach solution (3 oz bleach per gallon of water) or a 2.5% Quaternary ammonia (Sani-Care Quat-128, etc.) solution (3.5 oz quaternary ammonia per gallon of water). For absorbent materials , use a 2.5% Quaternary ammonia (Sani-Care Quat-128, etc.) solution (3.5 oz quaternary ammonia per gallon of water). Note: an alternative disinfectant can be used, if approved by the Project Manager.